Antifungal Activity of Type III Dental Gypsum Incorporated with 3-iodo-2-Propynyl-Butylcarbamate

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Abstract. The fungal growth on dental model can damage and affect the physical appearance of the gypsum. Fungi can be transferred among patients and dental personnel. Moreover, they relate to numerous illnesses. Thus, the development of antifungal dental gypsum is required to avoid the fungal growth on dental models. This study evaluated antifungal properties of 3-iodo-2-propynyl-butylcarbamate (IPBC) incorporated into type III dental gypsum. Three types of dental gypsum (Sirius, Ultima, France, 0.005% w/w IPBC and non-IPBC Siam Moulding Plaster, Thailand) were tested according to modified ASTM G 21-96 method with *Penicillium notatum* MI-311, *Aspergillus flavus* MI-321, and *Aspergillus spp.* isolated from orthodontic models. 50 μL of spore suspension of each fungus (10^4CFU/mL) was dropped on the prepared gypsum samples and incubated at room temperature, ≥85% relative humidity for 28 days. Fungal growth was visually scored. No fungal growth was observed on IPBC gypsum while 2 strains of *Aspergillus spp.* could be found on sirius gypsum. Type III dental gypsum incorporated with IPBC shows significant antifungal activity ($p < .001$) compared with non-IPBC and Sirius groups. This developed gypsum with IPBC can be used to fabricate dental models to prevent any damages from fungal growth.

1 Introduction

In orthodontics, type III dental gypsum is traditionally used to fabricate dental models, which required for diagnosis, treatment planning, communication, treatment record, and especially medicolegal purpose. Moreover, The American Board of Orthodontics (ABO) requires dental models to be smoothed and polished in such a manner that tooth and soft tissue details are not destroyed.

The growth of fungi is one of dental model storing problems. It does not only affect the physical appearance of the models but also can damage the gypsum [1], [2]. In addition, fungi associate with numerous health effects such as upper respiratory tract symptoms, cough, wheeze, respiratory infection, bronchitis, asthma and other problems [3], [4].

The isolated fungi from plaster dental models are *Cladophialophora spp.*, *Trichosporon beigelii*, *Aspergillus flavus* and *Aureobasidium pullulans* [5]. The sources of these fungi are from patient's secretion and environment.

The healthy oral cavity presents more than one hundred species of fungi. The most frequently detected oral fungi are *Candida spp.* (75%), followed by *Cladosporium spp.* (65%), *Aureobasidium spp.* (50%), *Saccharomycetales spp.* (50%), *Aspergillus spp.* (35%), *Fusarium spp.* (30%), and *Cryptococcus spp*. (20%) [6]. Furthermore, the predominant fungi isolated from environmental were *Aspergillus spp., Penicillium spp., Stachybotrys spp. and Cladosporium spp* [7], [8].

Using of fungal-resistant construction products that are incorporated with the antimicrobial material is one way to reduce the risk of fungal growth. There are many agents which can be used for this aim, such as copper montmorillonites, zinc montmorillonites, silver montmorillonites [9], silver zeolite, silver zirconium phosphate silicate, silver zirconium phosphate [10], azole compounds [11], and 3-iodo-2-propynyl-butylcarbamate (IPBC) [12].

IPBC has antimicrobial and fungicide properties widely used as a preservation in cosmetics, paint, inks, adhesives, plastics, paper, textiles, emulsions, metalworking fluids, and wood products. It can be used against several types of bacterial and fungi [13], [14]. The effective antimicrobial concentration of IPBC in type III dental gypsum, which has acceptable physical properties, is 0.005% w/w [15]. Thus, the aim of this study was to evaluate the antifungal properties of 3-iodo-2-propynyl-butylcarbamate (IPBC) incorporated into type III dental gypsum.

2 Materials and methods

2.1 Microorganism

Aspergillus spp. isolated from patient's orthodontic dental model and two types of fungi (*Penicillium*

notatum MI-311, *Aspergillus flavus* MI-321) obtained from the Oral Microbiology Department, Faculty of Dentistry, Mahidol University, Bangkok, Thailand were grown on Difco™ Sabouraud Dextrose Agar at 30 to 35°C for 7 days. After that, the tested strains were transferred into RPMI-1640 medium (with glutamine, without bicarbonate, and with phenol red as a pH indicator) (Sigma #R-7755 St. Louis, USA) (Life technologies™) supplemented with 0.2% glucose. Microbial suspensions were adjusted using a spectrophotometer with a tested inoculum in the range $0.4x10^4$ to $5x10^4$ CFU/ml with the optical density (OD) of 0.09 - 0.11 at 530 nm according to CLSI M38-A standard for molds[16].

2.2 Dental gypsum

In this study, type III dental gypsum were divided into 3 groups as follows non-IPBC group (Siam Moulding Plaster Co., Ltd, Thailand without disinfectant), IPBC group (Siam Moulding Plaster Co., Ltd, Thailand with 0.005% w/w IPBC), and Sirius group (Sirius, Ultima, France).

2.3 Antifungal testing

The antifungal testing was done according to the modified ASTM G 21-96 method[17, 18]. All groups of dental gypsums were prepared and poured into petri dishes and then remain undisturbed for 60 minutes to completely set. Fifty µl of each microbial suspension was dropped on the prepared dental gypsum samples. Petri dishes were sealed by laboratory sealing film and were incubated in the container for 28 days at 30 to 35°C and 85 % relative humidity. The density of fungal colonies on gypsum samples were examined under microscope and were scored at day 7, 14, 21 and 28 base on the fungal growth as shown in Table1.

All experiments were done in triplicate with 3 separate occasions. The fungal growth scores were carried out by the same researcher.

Table 1. Fungal growth scoring criteria

Growth	Score
None	0
Traces of growth (Less than 10%)	1
Light growth (10-30%)	2
Medium growth (30-60%)	3
Heavy growth (60% to complete coverage)	4

2.4 Intra-examiner reliability

To evaluate intra-examiner reliability, 10 samples were randomly selected from all tested petri dishes. Each selected sample was scored 3 times with the time interval of 1 week.

2.5 Statistical analysis

The fungal growth scores were analyzed to evaluate the antifungal activity of three groups of type III dental gypsum with the Fisher's exact statistical test at 95 % confidence interval; the fundamental significance level was thus set at $\alpha = 0.05$. The intraclass correlation coefficient (ICC) was performed to evaluate the intra-examiner reliability.

3 Results

Almost perfect intra-examiner reliability was determined (ICC = 0.973). There is only one error of intra-examiner fungal growth scoring from score 1 to score 0.

The median and range of fungal growth scores of each group are demonstrated in the Table 2. No fungal growth was observed on Type III dental gypsum incorporated with IPBC under the experimental conditions. Whereas, the median values of fungal growth scores of *P. notatum*, *A. flavus,* and *Aspergillus spp.* isolated from models in non-IPBC group were 4, 3, and 4 respectively. In Sirius group, the median values of fungal growth scores similar to non-IPBC group's scores except *P. notatum* that showed no growth. Thus, type III dental gypsum incorporated with IPBC exhibited significantly better antifungal activity against all tested fungi than non-IPBC and Sirius groups ($P < .001$).

Table 2 . Fungal growth scores on 3 types of dental gypsum.

Fungi	Type III dental gypsum		
	Non-IPBC	IPBC	Sirius
Penicillium notatum	4 (3-4)	0 (0-0)	0 (0-0)
Aspergillus flavus	3 (3-4)	0 (0-0)	3 (3-4)
Aspergillus spp. isolated from model	4 (3-4)	0 (0-0)	4 (3-4)

*Data expressed as median value and (range) of 9 replicates.

4 Discussion

Cross contamination is a major problem in dentistry. Several harmful microorganisms which come from oral cavity and environment [5], [19] can be spread and transferred among patients, dental health personnel, and technicians in dental clinic and laboratory via dental models. For example, orthodontic appliances such as retainer are fabricated on dental models before put on patients. If dental models are contaminated with fungi, they can transfer to dentists and patients during treatment procedures.

Table 3. Fungal growth scores of each sample at time interval.

Fungi	Type III dental gypsum																										
	Non IPBC									IPBC									Sirius								
	A			B			C			A			B			C			A			B			C		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Penicillium notatum																											
7 d	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14d	3	3	3	2	2	2	2	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21d	4	4	4	3	3	3	3	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28d	4	4	4	4	4	3	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus flavus																											
7 d	3	2	2	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	3	2	3	0	1	1	1	2	1
14d	3	3	3	2	2	2	1	1	1	0	0	0	0	0	0	0	0	0	4	3	4	1	2	2	2	2	2
21d	4	4	3	3	3	3	2	3	3	0	0	0	0	0	0	0	0	0	4	3	4	2	3	3	2	3	2
28d	4	4	3	3	3	3	3	4	4	0	0	0	0	0	0	0	0	0	4	4	4	3	3	3	3	4	3
Aspergillus spp. from model																											
7 d	3	2	3	3	3	3	3	3	2	0	0	0	0	0	0	0	0	0	1	1	1	3	3	2	0	1	1
14d	3	2	4	3	3	3	3	4	3	0	0	0	0	0	0	0	0	0	2	1	2	3	3	3	1	3	2
21d	4	3	4	4	4	4	4	4	3	0	0	0	0	0	0	0	0	0	3	3	3	4	4	4	2	3	3
28d	4	3	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	4	4	4	4	4	4	3	4	4

d stands for days. a, b and c are three replicas. A, B and C are three occasions.

Furthermore, fungi can damage gypsum of dental models by creating the decay from both physical and chemical actions [20]. Only small decay on dental models can impact the analysis activities such as diagnosis, treatment planning, and treatment evaluation in orthodontics because the analysis of tooth alignment, dental arch perimeter, and occlusion in orthodontics require all details at millimeter-level from dental models. Therefore, dental gypsum containing antimicrobial agents can be one of methods to solve these problems.

3-iodo-2-propynyl-butylcarbamate or IPBC is used as preservative agent in various products. In this study, the incorporation of IPBC into type III dental gypsum demonstrated effective antifungal activity against all tested fungi compared with non-IPBC and Sirius groups of dental gypsum. However, the mechanism of IPBC antimicrobial activity is not clearly understood. IPBC has approximately 1,000 times more toxic to invertebrates and fish than the propargyl butyl carbamate (PBC), primary hydrolysis metabolite without iodine. Thus, a possible mechanism may be related to the iodine [21].

Iodine is a broad-spectrum antimicrobial agent that has simultaneously action against gram positive, gram negative, spores, amoebic cysts, fungi, protozoa and yeasts, and Methicillin-resistant *Staphylococcus aureus* (MRSA). It affects enzymes of respiratory chain, lipid membrane, and nucleic acid function that interferes with the permeability of the cell membranes and denatures protein [22]. Thaweboon's study showed the antimicrobial activity of type III dental gypsum incorporated with IPBC against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* [15]. However, IPBC is degraded by some strains of proteobacteria (Alcaligenes, Enterobacter, Pseudomonas, Ralstonia, etc.) [23].

In term of safety profile, the 2013 Cosmetic Ingredient Review (CIR) Expert Panel concluded that the safety concentration of IPBC for cosmetic is ≤ 0.1%. There are low risks of contact sensitization and allergic contact dermatitis at this concentration level. Moreover, IPBC neither has embryotoxic, teratogenic, mutagenic, nor carcinogenic effects [24]. Therefore, the amount of IPBC used in dental gypsum (0.005% w/w) is far below the threshold of safety limit for that used in cosmetics.

From Table 2 and Table 3, dental gypsum in Sirius group also showed some antifungal activity against *P. notatum* and retarded growth of *A. flavus* and *Aspergillus spp.* isolated from models. In accordance with information technique - technical datasheet of Sirius [25], this type of gypsum contains additives to protect absorption of moisture which is one of important factors influencing the growth of fungi on the materials [26].

In dentistry, gypsum models of patients' dentition are one of recognized tools for orthodontic diagnosis and treatment planning as well as medico-legal purpose. Dental models are the traditional three-dimensional patient record for measuring linear changes in the dentition during treatment and, therefore, have to be stored for many years. In our study, antifungal property of dental gypsums was evaluated during the period of 28 days according to the ASTM G 21-96 antifungal testing

method for materials. This might be the time limitation of our study.

Concerning other properties of studied dental gypsums, even though the physical properties of IPBC incorporated dental gypsum were not determined in this study compared with others, data from our previous study have shown that this type of dental gypsum has satisfactory physical properties within the ISO standard of dental materials [15]. Further investigation on other contaminated fungi and the sustained period of antimicrobial action of this modified dental gypsum are still required.

As demonstrated in experimental results, Type III dental gypsum incorporated with 3-iodo-2-propynyl-butylcarbamate (IPBC) had no fungal growth whereas other two types of gypsum had medium to heavy fungal growth. Therefore, we can concluded that Type III dental gypsum incorporated with IPBC had antifungal effect on *P. notatum*, *A. flavus* and *Aspergillus spp.* isolated from model at 30 to 35°C, 85 % relative humidity for 28 days. This developed antifungal dental gypsum could be used to fabricate dental models, which can be preserved overtime for the purpose of treatment planning and evaluation. In addition, further study will be conducted with other fungal species and microorganism such as *Cladophialophora spp.*, *Trichosporon beigelii*, *Aureobasidium pullulans,* coagulase-negative *Staphylococcus, Micrococcus spp*., *Bacillus spp*., and nonfermenting gram-negative bacillus [5] to investigate the effectiveness of Type III dental gypsum incorporated with IPBC regarding antimicrobial property.

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